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NUTRITION REVIEWS

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FOOD FADDISM—WHY?

Food faddism, which is economically wasteful, scientifically unsound, and a source of great distraction to nutritionists, physicians, and health agencies working to promote better nutrition education, persists and grows. We might inquire of ourselves how this can be at a time when scientific knowledge about nutrition is highly developed and current research is contributing additional knowledge daily.

Food faddism persists, I believe, despite incontrovertible scientific evidence denying its validity, because food has emotional rather than intellectual value to the average person, and the food faddists have capitalized upon this fact, appealing to the emotional drives of their believers rather than to their intellects.

Knowledge of the emotional value of food is of course much older than the knowledge of its nutritional value, both in the historical and in the individual biological organism. One of the primordial human urges is the urge to eat. In prehistoric times food was obtained solely to satisfy this urge. In our own modern civilization, with scarcity of food no longer a major problem, food is still meaningful as a symbol of acceptance, friendliness, and socialization. As Harriet Moore has pointed out, (*Am. J. Clin. Nutrition*, **5**, 77 (*1957*)) we tend to accept food best from those we consider our friends, we tend to distrust food given us by outsiders, and we may reject entirely the fare of strangers, or persons from a markedly different environment or culture. Food may be considered masculine (meat), feminine (vegetables, fruits), or neuter (eggs, fish, coffee). Food may be looked upon as a sign of emotional and cultural identification, as a badge of prestige and social position, and even as a weapon; and it is visualized by most persons in these contexts rather than as a source of nutrients for growth and metabolism. Studies suggest that the infant, in his earliest periods of consciousness, regards food as being equated with love, pleasure, protection, and comfort. Other studies support the view that this feeling may even persist in the adult, and, under pathological conditions, may be the basis of the drive to overeat.

The food faddist appreciates the symbolic value of food better than those of us concerned with scientific nutrition. Each person has his own galaxy of food-associated emotional half-tones, many of them unconscious. Although intelligent nutrition education must be predicated upon the truths of scientific nutrition, the blandishments of the "health" food educator may be based solely upon emotional responses to food as a symbol. Hope, fear, and superstition play a large role in the winning of adherents. The astonishing success of this latter approach reminds us that no program of nutrition education can be effective if the emotional aspects of food and diet are ignored.

Food faddism is of two general kinds. The first is individual, while the second is collective. The former generally stems from acute or chronic psychological aberration, including psychosis. The latter comes about through acceptance by a group of stereotyped dietary practice designed to improve health or cure disease. The latter may be cultural, or it may be stimulated for profit by a smooth-talking, pseudo-sophisticated, high-pressure salesman with a minimum of training in nutrition and a maximum of various important professional associations and degrees.

Into the first category of individual faddism may be placed the odd dietary practices indulged in during pregnancy as represented by cravings for cornstarch, clay chalk, charcoal, or tree bark. "Gourmet" leanings to kangaroo tails, fried grasshoppers, baby bees, and pickled ants may be tell-tale of a food neurosis. In psychotic or schizoid individuals a devotion to fasting as

a method of "improving the mind" or "eliminating impurities" has been noted by many. Marked swings of appetite leading to cyclic manifestations of obesity and anorexia nervosa have been noted in a single disturbed individual. (H. W. Brosin, *New Engl. J. Med.* **248,** *974 (1953)*).

Into the second category go the innumerable food fallacies subscribed to by various cults or groups. Most of these faddists, I believe, should be considered psychologically exploited rather than psychiatrically ill. Several specific erroneous beliefs about food widely subscribed to by such faddists come to mind. One is the fallacy that ascribes to a single food, or group of foods, a special attribute or power which is out of proportion to its nutritional content. Some foods, widely promoted in this manner, include yoghurt, blackstrap molasses, wheat germ oil, brown sugar, "live" honey, and seaweed.

Another fallacy states that currently available fresh fruits and vegetables are nutritionally barren because most of them are grown with chemical fertilizers. Only that produce which is grown from fields with "organic" fertilizers is said to be nutritionally adequate. Exhaustive biochemical analyses, however, have failed to reveal any significant differences in the content of a wide range of nutrients per unit weight of crops grown under the two conditions.

A third fallacy is that which advocates the daily fortification of the American diet with vitamins, trace minerals, and other nutrients on grounds that such fortification will improve health beyond that of the usual adequate diet. This fallacy is particularly appealing because it is built upon the insurance premise of "the more the better." No evidence is available to support the view purporting that because a nutrient at suboptimal levels in the diet causes disease or disability, the same nutrient at supra-optimal levels improves health beyond the optimum. There is good evidence, on the other hand, that some nutrients may actually cause nutritional imbalance and toxicity if given in large excess. Fortunately, this is not true of the vitamins of the B-complex, when given to well-nourished individuals. Taken routinely by a large segment of our population, the B-complex vitamin capsule has become the great American placebo.

The fourth fallacy, more damaging to health than the former, is the belief that certain foods or systems of dieting have specific value out of proportion to the established facts of diet therapy in the treatment of given diseases. A host of "elimination" regimens to promote diuresis or defecation have been popularized to "cure" hypertension, cancer, heart disease, diabetes, anemia, and obesity. A recent best-seller advocated a system of completely unfounded dieting for the cure of rheumatoid arthritis. Another group of cultists have emphasized the taste, consistency, and extent of mastication of the food as the critical factor in the promotion of health. Numerous regimens for weight reduction, including such restricted menus as (1) bananas and milk, (2) steak and prunes, and (3) raw tomatoes and hard-boiled eggs, have been advocated as being *specifically* useful for the treatment of obesity. Many such diets are nutritionally inadequate as well as being calorically inappropriate.

Recently much attention has been given to the possibility that low-fat low-cholesterol diets were useful in the lowering of serum cholesterol, and possibly in improving the prognosis in coronary artery disease. There is some evidence to support this type of diet therapy in *selected* individuals, but the extent to which this mode of dieting has won adherence by patients, some not under a physician's care, is approaching a faddism. In all cases careful follow-up by a physician to determine the effects of such diet therapy upon serum cholesterol and cardiovascular status is mandatory.

In conclusion, we may query, "How can food faddism be more effectively combatted?". I believe that there are two main avenues of approach to this problem. The first is an effective program of nutrition

education in which the facts about nutrients, food values, diets, and diet therapy are presented in an enlightened and appealing manner so as to convey sound nutrition information without minimizing the symbolic value of food. The second is to improve the standards of the practice of clinical nutrition by physicians in our country. The family physician should be the first court of appeals in matters of health. Unfortunately, many practitioners of medicine are not fully informed in the field of nutrition and diet therapy, and hence are not prepared to consult with patients about the faulty diet practices which constitute the basis of faddism.

Clinical nutrition is a specialty of medicine. It should be practiced by physicians and other allied professional personnel, rather than by high-pressure salesmen. It is our responsibility to see that this becomes a reality through improved teaching of nutrition in medical school and in post-graduate training activities. When this is accomplished, I believe that the grip which food faddism has upon the American public will lessen greatly.

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ELECTROLYTE DISTURBANCES IN CHRONIC MALNUTRITION

The continuing high mortality in severe chronic infantile malnutrition in Mexico City occurs largely during the first 24 to 48 hours after hospital admission, and has been attributed to electrolyte disturbances. The intracellular composition and homeostatic mechanisms in severe chronic malnutrition have been the subject of a collaborative study by the Pediatric Departments of the Hospital Infantil de México and the Children's Hospital of Boston (F. Gómez *et al.*, *Pediatrics* **20,** *101 (1957)*).

These authors point out that electrolyte imbalances occurring in chronic malnutrition should not be evaluated according to concepts derived from studies on well-nourished infants. They point out that the chronically underfed child gradually becomes adapted to his metabolic status by slowing, or almost complete, cessation of growth. Bouts of diarrhea recur and eventually the acute depletion of fluid and electrolytes may overwhelm the child whose natural defenses have been disturbed by chronic metabolic changes.

Dehydration is one of the most prominent changes occurring in this group of patients. The dehydration is associated with hypotonicity of the body fluid, a lack of thirst and, in the more severe cases, an increase rather than a decrease in urine output. The child whose intake of water has been relatively excessive, compared with his low intake of sodium and potassium, has developed a response to dehydration which has been described as "functional diabetes insipidus," although it could be demonstrated that the kidney was able to respond to antidiuretic hormone in the normal manner. Potassium deficiency is a prominent feature in this condition and may not be accompanied by alkalosis, which characterizes potassium deficiency in previously well-nourished children. The usually recommended therapeutic intakes of potassium may prove to be insufficient.

Tissue composition was determined by analyses of skin and muscle biopsy material in 22 patients (S. Frank, *et al. Pediatrics* **20,** *105 (1957)*). One-third of these patients had edema and four of them had extreme wasting. The composition of muscle was characterized by an increase in the content of water per unit of dry, fat-free solids. The increase in water was both extra- and intra-

cellular except in the "atrophic" group showing extreme wasting, in which the volume of intracellular water was relatively normal but was often less than the volume outside of the cells, a reversal of normal relationships. The potassium content of the muscles was not greatly decreased except in the edematous children. The intracellular sodium was generally increased. The interrelationships between sodium and potassium within the cells did not follow usual patterns and suggested that a disturbance of the osmotic properties of the muscle cells was present.

The skin showed an increased content of water, sodium and chloride despite clinical dehydration in some cases. The edematous subjects tended to have the greatest amount of extracellular fluid in the skin, although similar values were found in cases that were not edematous. In the latter subjects there was a great reduction in subcutaneous fat. These observations would suggest that a certain amount of subcutaneous fat was necessary for the appearance of clinical edema, and also that the presence of edema indicated a more acute depletion in a child not as severely malnourished as in one who had lost his subcutaneous fat.

Preliminary results on the study of renal function in these patients (G. Gordillo, *et al.*, *Pediatrics* **20,** *303* (*1957*)) indicated that the glomerular filtration rate (inulin clearance) and renal plasma flow (clearance of para-aminohippurate) were both significantly reduced. This reduction was most evident during periods of clinical dehydration. A decrease in osmolar clearance suggested that mechanisms designed to conserve sodium were operating effectively. At the same time these patients excreted "free water" despite the demonstration that antidiuretic mechanisms were intact. These observations suggested that the kidney was conserving the concentration of electrolyte in the body fluids at the expense of an increase in volume. The authors further suggested that the clearance of "free-water" might constitute a method for maintaining the normal volume of the body fluids.

These extensive and detailed observations have been summarized and interpreted in a final paper in this series (J. Metcoff, *et al.*, *Pediatrics* **20,** *317* (*1957*)). Serial biopsies of skin and muscle and numerous balance studies and renal function tests have suggested at least one pattern in the development of the electrolyte alterations in chronic malnutrition. Extracellular hypotonicity with expansion of the volume of intracellular water occurs irrespective of volume changes in the extracellular fluid compartment. This increase in intracellular water is accompanied by accumulation of sodium within the cells quite independently of the content or concentration of potassium. This would indicate that either the osmotic properties of the cell or the cell metabolism, or both, were altered. This progression of events was reversed during recovery.

Evaluation of various therapeutic regimens was attempted using continuous renal function and balance studies. In several cases a redistribution of the body water with a partial restoration of normal conditions was achieved by the use of relatively hypertonic solutions. However, the indications and limitations of such therapy have not yet been defined adequately to permit its routine use.

Death, when it occurred, was attributed to the stress of an episode of diarrhea or infection which overcame the ability of the cells to respond in their chronically depleted state.

Although these careful and detailed studies cannot, at present, be used as a background for specific regimens in the treatment of the electrolyte disturbances frequently accompanying chronic malnutrition, they have given us information which would appear to establish the fact that these alterations are quite different from those found in well-nourished subjects suffering from similar disturbances. Emerg-

ing from these considerations is the observation that chronic malnutrition for both protein and calories has profound effect upon the cellular and regulatory mechanism of the body. Adequate recognition and treatment of these disturbances would appear to play a very important role in the treatment of chronic malnutrition.

EVALUATION OF SKELETAL AGE IN ROENTGENOGRAMS

Roentgenographic evaluation of skeletal maturation has become an accepted technique in the assessment of the development of children and state of health. A common technique is the comparison of X-rays made under standard conditions with reproduction of films of normal children carefully selected as norms for certain ages (Todd, T. W., *Atlas of Skeletal Maturation—Hand, St. Louis, C. V. Mosby (1937)*; W. W. Gruelich and S. I. Pyle, *Radiographic Atlas of Skeletal Development of Hand and Wrist, Stanford, Stanford University Press, (1950)*). This method of comparison has come into widespread use primarily during the school ages. The appearance of ossification centers is of considerable use in assessing the skeletal age of small infants and young children, and the use of epiphyseal fusion is valuable only in the adolescent group.

Despite the care that has gone into the selection of standard films and their careful reproduction in the atlases, there has been little study of the error inherent in the assessment of X-rays for skeletal age by using the inspectional method of comparison. The systematic errors in such assessments have been evaluated by D. Mainland (*Pediatrics* **12,** *114 (1953)*). The actual size reproductions of 90 hand roentgenograms from 24 children appearing in Macy's *Nutrition and Chemical Growth in Childhood, Vol. 2, Springfield, C. C. Thomas (1946)* were assessed in as an unbiased manner as possible by one experienced, but not expert, observer. Her assessments were compared with those made by Todd, using his atlas, and those made by Pyle, using the Gruelich-Pyle Atlas which is published in volumes 2 and 3 of the work by Macy.

The resulting observations were analyzed statistically. It was established that the assessor had developed a stable system of reading the *X*-rays and was shown to have an average systematic error of approximately two months less than that of the expert evaluations of the same films with a 95 per cent probability range of from -6 to $+3$ months. The systematic error of this observer varied significantly between roentgenograms from the same child and further significant differences between children were found. No cause for these differences in systematic error was apparent.

Supposing that the systematic error of a single assessor was approximately three months with a standard deviation of seven months, Mainland suggests that the unbiased assessment of skeletal age on a single film would prove to be too unreliable for general use. If there were 100 films in the test series, they would give useful results because the sample size would compensate for the individual variation.

The variable error of an observer in skeletal age assessment of hand roentgenograms (*i.e.*, the irregular variation between independent readings of the same film) has been reported on in more extensive material (Mainland, *Pediatrics* **13,** *165 (1954)*). The variable error for the observer referred to above was expressed by a standard deviation of approximately three to four months. This would require an assessor to affix an error of plus or minus 8.3 to 11.1 months to any single observation in order to obtain 95 per cent confidence limits. There was no significant difference in variable error associated with the atlas used, age, sex, difference between skeletal and chrono-

logical age, differences between children, or differences between X-rays of the same child. Methods are outlined in these two articles which would allow an individual assessor to measure both his systematic and variable errors in interpreting X-rays for skeletal age.

The differences between assessors, between assessments of the same film by the same assessor after a short interval (variable error) and systematic change in an assessor standards during a year's lapse of practice stimulated Mainland (*Ibid.* **20,** *979 (1957)*) to attempt to devise a more quantitative assessment than the inspectional comparison of individual films with a standard atlas. The measurements made on films of the wrist consisted of the oblique axes of the epiphyses of the radius, capitate, third metacarpal, and proximal phalanx of the third digit. In addition, the width of the shaft at all the epiphyses was measured as well as the distance from the radius to the third metacarpal as an estimate of carpal length. These measurements were made with fine dividers applied to a steel scale with a vernier reading to 0.01 cm. These measurements were applied to the material discussed above. The accumulated data were thoroughly explored statistically. An estimation of skeletal age by moving average curves and by multiple regression equations was attempted with various adjustments for hand size and different weighting of maturity indicators.

By using the simple measurements of a few maturity indicators, Mainland showed that it was possible to obtain estimates of skeletal ages that were equivalent to inspectional estimates in the study of groups of children between the age when ossification centers have appeared and the age of incipient epiphyseal fusion. The differences between the measurement estimate and the inspectional estimate varied so greatly from one film to another that the measurement method seemed to be of little use in assessing skeletal age or progress of individual children. The inspectional assessments were also very crude when used for this purpose. Even the "actual-size" reproductions in the preparation of an atlas was found to cause a change in size and consequently comparisons with the standard atlas had to be made on copies of the original films.

This very thorough review of the errors involved in estimating skeletal age from roentgenograms emphasizes that the variable and systematic errors for any observer should be determined before he makes assessments of films to be used in a nutrition or growth study. The techniques for these procedures are clearly outlined. Even with adequate knowledge concerning both of these sources of error, it would appear that this technique is applicable only to relatively large groups of 50 to 100 or more individuals, and that assessment of skeletal age from X-ray films is not particularly reliable in individual instances. In actual medical practice, however, comparison of films with those taken previously and consideration of the very great magnitude of aberrations found in disease do not make this approach wholly unreliable from a diagnostic standpoint.

PLASMA UNESTERIFIED FATTY ACIDS

Interest in the unesterified fatty acids of plasma has been considerably increased by the publication of the papers of Dole and his associates, and those of Gordon's group in 1956 and 1957 (see below). The work is an outgrowth of investigations on the lipemia-clearing action of heparin (*Nutrition Reviews* **12,** *294 (1954)*), which produces a concomitant rise in plasma fatty acids. This is thought to result from the release of a lipase which hydrolyzes plasma fat, with decrease in lipemia and removal of fatty acids to the tissues. As a result of the work reviewed here, the fatty acids have assumed

greater importance than was earlier realized (*Ibid.* **14,** *216* (*1956*)). Their metabolic relations to diabetes and fat deposition, transport and utilization are under study. The rapidity with which the plasma fatty acid concentration varies attests to a very labile metabolic pool in which, indeed, recent isotopic studies indicate an extraordinarily rapid turnover.

V. P. Dole (*J. Clin. Invest.* **35,** *150* (*1956*)) found that the plasma level of non-esterified fatty acids was not affected by a fatty meal, but that it decreased after a mixed meal. This could be duplicated by feeding glucose or injecting insulin. Fasting caused a slow rise; epinephrine caused a prompt and marked rise. In a paper published at the same time, R. S. Gordon, Jr., and A. Cherkes (*Ibid.* **35,** *206* (*1956*)) also found a slow rise during fasting, a two hour fall after glucose, and a consistent rise at three to four hours after glucose, suggesting an epinephrine effect. They documented this, showing a peak rise at the end of a 20-minute infusion containing 1 mg. epinephrine, and a return to the starting level by 60 minutes.

R. S. Gordon, Jr., with the assistance of A. Cherkes and H. Gates (*Ibid.* **36,** *810* (*1957*)), has studied the unesterified fatty acid in human plasma with reference to its transport function. These workers lyophilized plasma samples, extracted the powder with a glacial acetic acid-isooctane mixture and titrated with sodium hydroxide, using Nile Blue as an indicator. Suitable controls established the sensitivity and specificity of the method. In particular, it does not measure pyruvate, lactate or beta-hydroxybutyrate; the authors consider it more specific than previous methods.

The subjects for the study were fasting patients undergoing cardiac catheterization for the diagnosis of heart disease, and normal conscientious objectors. By measuring concentrations in arterial and in coronary sinus or hepatic vein blood, the authors could demonstrate extraction of unesterified fatty acid from the blood perfusing the myocardium and enteric circulation, respectively. When saphenous blood concentrations were measured, they proved *higher* than the arterial levels by six to 48 times the standard error of the method. Thus fasting concentrations in arterial blood ranged from 0.50 to 0.87 mEq per liter in five subjects, while venous levels were 0.77 to 1.80 mEq per liter. The saphenous vein blood was presumed to drain principally subcutaneous tissue. Similar measurements on arterial and venous blood samples "from the forearm muscles" showed positive arteriovenous differences in 15 of 22 cases (indicating extraction by muscle), negative in four, and inconclusive in three. The figures are not given. When renal extraction was measured on appropriate samples, the arteriovenous differences in nine patients were from plus 0.08 to minus 0.03 mEq per liter, too small a difference to be significant.

The authors studied arteriovenous differences (hepatic and saphenous veins, three cases each) before and after an intravenous dose of insulin with glucose (0.1 unit per kilogram body weight and 100 g., respectively). In all samples (venous and arterial) there was a sharp reduction in unesterified fatty acid concentration and in each experiment the arteriovenous difference (positive for the hepatic, negative for the saphenous blood) was abolished.

In another series of experiments, changes in unesterified fatty acid concentration of antecubital vein blood were measured after feeding various nutrients in 300 ml. water to eight normal fasting subjects. The advantages of arterial sampling were eschewed because of the discomfort of the procedure. The following were studied, on different days: water alone, 300 ml.; glucose, 50 g.; DL-alanine, 50 g.; L-glutamic acid, 50 g. and L-leucine, 25 g. The results were graphed as changes from the fasting level, averaged for the eight subjects. The normal fasting trend for unesterified fatty acid was a straight line rising almost 0.2 mEq per liter in three hours. All the nutrients reversed this for the first two hours, the amino acids producing a nadir of about minus 0.05 to minus 0.1 mEq per

liter and glucose about minus 0.25. At three hours the levels were all above initial fasting levels, but below those of the subjects fasted for three hours.

Gordon, Cherkes and Gates made a very interesting calculation showing that myocardium may well derive a considerable part of its energy from fatty acid oxidation. Using an assumed molecular weight, they calculated the amount of fatty acids extracted by the myocardium and computed the amount of oxygen required for its combustion. This came to 36 to 87 per cent of the total myocardial oxygen consumption as determined by the arteriovenous oxygen differences for the heart. The ratio for hepatic vein blood was 31 and 103 per cent in two cases.

The authors conclude that the only likely explanation for the negative arteriovenous differences found with saphenous blood is that fatty acids enter the circulation from adipose tissue. Similarly, their evidence indicates extraction of these acids from the blood by myocardium, the viscera drained by the hepatic vein and, probably, skeletal muscle. They present calculations to show that the large renal flow in relation to metabolic demands of the kidney would make the maximum possible arteriovenous differences about 0.05 mEq per liter, close to the standard error of the method (0.02 mEq per liter).

The hepatic vein studies, as the authors point out, are complicated by the fact that this blood includes that coming from omental and other visceral adipose tissue. Adding to this complication, it includes hepatic and visceral outflow in a mixture one hopes future work will separate. A fascinating array of potential findings awaits the investigator who measures differences between fatty acids in portal and hepatic venous as well as arterial blood. The changes in liver extraction or synthesis vis-a-vis adipose tissue and muscle, as affected by fasting, glucose and fatty meals in the diabetic patient and the normal individual after administration of various hormones, will amplify considerably the observations already recorded.

Dole (*Fed. Proc.* **16,** *11* (*1957*)), using C^{14}-labeled palmitic acid in acute rabbit experiments, showed the expected decrease in fatty acids after insulin injection. But the rate of disappearance of the C^{14}-palmitic acid was not altered by insulin. He inferred that insulin limits the release of free fatty acids from the tissue stores rather than accelerating their removal from plasma. His work with isotopic tracers indicates that plasma non-esterified fatty acids have a half-time of only a few minutes; this explains the rapid changes in their concentration with any change in turnover rate (*J. Clin. Invest.* **36,** *884* (*1957*)). Diabetic subjects showed a delayed and decreased or absent fall in plasma fatty acids when given oral glucose or glucagon. Insulin caused a prompt fall, hours before disappearance of ketonuria and ketonemia. Fructose also caused a prompt decrease. Tolbutamide caused a fall in glucose and fatty acids in normal subjects or stable diabetic individuals, but not in juvenile diabetic patients with histories of repeated ketosis. Insulin caused a prompt decrease in both. Dole concludes that utilization of carbohydrate inhibits release of non-esterified fatty acids from fat depots and that this mechanism is faulty in unstable diabetic patients.

In further work, E. L. Bierman, T. N. Roberts and V. P. Dole (*Proc. Soc. Exp. Biol. Med.* **95,** *437* (*1957*)) showed that tolbutamide, given "to normal and diabetic patients caused a reduction of non-esterified fatty acid concentration only in those subjects who responded with hypoglycemia". They consider that their findings support the theory that tolbutamide stimulates insulin secretion and does not directly increase glucose oxidation.

D. A. Dreiling and E. L. Bierman (*Ibid.* **95,** *496* (*1957*)) found that "blood amylase and plasma non-esterified fatty acid concentrations responded in a correlated way to glucagon, glucose, fructose, epinephrine and orinase" (tolbutamide). "Apparently these

two components are associated in some common metabolic process, extrapancreatic in location".

In attempting to explain the known facts about plasma fatty acid transport, Gordon, Cherkes and Gates conclude that the abolition of arteriovenous differences after administration of glucose and insulin corresponds with "complete cessation of net transport" of unesterified fatty acids. They believe transport of fat in this form is controlled by the adipose tissue under the influence of different sources of calories, glucose and amino acids. The availability of calories from non-fat sources would be the chief determinant of plasma fatty acid concentration, through some quite unidentified receptor mechanism. The action of epinephrine would be an exception.

Dole's explanation appears simpler and more in harmony with the known facts of carbohydrate and fat metabolism, namely and simply that carbohydrate utilization inhibits the release of non-esterified fatty acid from fat depots. This is consistent with the observations of both groups on the effects of glucose, fructose, insulin, glucagon, tolbutamide, epinephrine and amino acids, and the known facts of metabolism in diabetic acidosis.

Once again we are presented with a picture of astonishing activity in a tissue (adipose), formerly thought relatively quiescent in its metabolism. Although the prompt availability of depot fat as a source of energy during brief or long periods of caloric insufficiency has long suggested this, the rapid changes in unesterified fatty acid concentration in the blood indicate an even greater turnover.

The findings of Dole's and Gordon's groups complement each other and are entirely consonant. The particular contribution of the latter is to quantify the amount of fatty acid transported by determining arteriovenous differences. Not only does the plasma level of a substance give no clue as to the size or turnover rate of a pool, but the direction to or from a depot is not apparent. Thus the work of Gordon's group indicates the amount and direction of transport, while information as to turnover rate will be most accurately provided by the isotopic tracer studies of Dole and his colleagues.

In summary, the unesterified fatty acids in plasma are extraordinarily sensitive to administration of glucose, insulin, epinephrine and other determinants of carbohydrate utilization, and insensitive to fat ingestion. They have a half-time of only a few minutes. In the fasting state they are extracted by a variety of tissues, but are put into the circulation by adipose tissue, as shown by arteriovenous differences. Both extraction and release are promptly stopped by administration of insulin and glucose.

METHIONINE AND URINARY AMMONIA

Within the past few years, two investigators reported that methionine overcame the unpleasant odor and debilitating skin rash seen among some infants and many incontinent patients. In all of these cases, the offending odor and, presumably, the skin irritant was reported to be ammonia which was formed after the urine was voided. The presence of ammonia in urine is usually ascribed to bacterial action unless the subject is in an acidotic state when endogenously produced ammonia is used for the neutralization of the keto acids.

The first of these reports came from L. Goldstein (*Arch. Pediat.* **66,** *553 (1949)*) who found that a fairly large number of infants on a pediatric ward developed inflamed areas on the thighs, genitalia, buttocks and lower abdomen when the urine smelled strongly of ammonia. The inflammation might be followed by ulceration if permitted to go untreated. The "napkin" or "diaper"

dermatitis as the above condition is called was associated with the excretion of a highly colored, acid urine.

Goldstein (*loc. cit.*) supplemented the formulae of 500 infants showing ammoniacal urine with 0.2 g. of DL-methionine (suspended in a dextrose, lactose mixture) per day for infants under one year of age and 0.3 to 0.4 g. for infants from one to two years of age.

All but three of the 500 infants showed a disappearance of the ammoniacal odor from the urine in three to five days. With this went a disappearance of the dermatitis and ulceration, and complete healing in seven to ten days. Of the three infants who failed to respond to the methionine, one refused the formula, the second vomited the formula, and the third developed a dermatitis suggesting a sensitization reaction.

On the basis of the above report, M. Bergman (*Geriatrics* **12,** *386 (1957)*) tried the addition of methionine to the diets of individuals in a mental hospital. A fairly large percentage of the patients in the hospital were incontinent. Associated with this was the unpleasant odor of the ward and the development of a skin condition which required extra nursing care in a hospital where such care was already at a premium. The addition of 0.6 g. of DL-methionine per day to the diet of 16 female and 70 male patients resulted in a "decided reduction in odor" and a "noticeable improvement in skin inflammation and ulceration."

As a control measure, methionine supplementation was stopped, resulting in the reappearance of the ammoniacal odor and the dermatitis within three days.

For some patients, the methionine supplement had to be raised to 0.8 g. a day to secure improvement in the skin condition. When the supplement was lowered to 0.4 g., there was no noticeable change in either the odor of the urine or in the condition of the skin.

These effects of methionine are interesting from a patient-care standpoint. It was pointed out by Bergman (*loc. cit.*) that the odor prior to the use of methionine was frequently so strong that hospital personnel quit their jobs. The observations, however, raise the question as to whether the effect is specific for methionine and whether it is in any way related to a disturbance in protein metabolism. Both of the above authors emphasize the possible role that methionine may have in protein metabolism and feel that its beneficial effect is due entirely to a correction in protein metabolism. Whether this is so will require additional investigation, since one is more likely to think of an antibacterial action of the methionine (or some compound derived from it) or a change in the pH which inhibits the growth of the ammonia-producing bacteria. Goldstein (*loc. cit.*) stated that he had tried choline and creatine, both of which were ineffective in preventing the formation of ammonia in the urine. On the other hand, thiamine at a daily level of 60 mg. per infant was effective.

Although the above effects are probably not related to any change in the nutritional status of the subjects, they do indicate that some dietary components may play important auxiliary roles. If further work were done on this problem, it might be well to carry out tests to determine how the methionine and thiamine inhibit ammonia formation in the urine. Such studies may suggest compounds that will do the same thing but at a lower cost.

NUTRITIONAL REQUIREMENTS OF FISH

Investigations into the nutritional requirements of fish have thus far been relatively limited. The recent extensive work in designing appropriate diets and in pursuing the effects of vitamins and amino acids in the diet of chinook salmon by J. E. Halver, U. S. Fish and Wildlife Service, Salmon Nutrition Laboratory, Cook, Washington, are there-

fore of considerable interest (*J. Nutrition* **62,** *225 (1957)*).

The composition of the vitamin test diet was as follows (parts): vitamin-free casein, 54; gelatin, purified, 15; corn oil, purified, 9; white dextrin, 8; alpha-cellulose flour, 9; mineral mixture, 4; DL-methionine, 1; and L-tryptophan 0.5.

The minerals consisted of U. S. P. XII Salt Mixture No. 2 which was fortified with the following (mg. per 100 g.): aluminum chloride, 18; zinc sulphate, 357; cuprous chloride, 11; manganous sulphate, 80; potassium iodide, 17; and cobaltous chloride, 105.

The test diet was also fortified with the following vitamins (mg. per 100 g.): thiamine-HCl, 6; riboflavin, 20; pyridoxine-HCl, 4; niacin, 80; Ca-pantothenate, 28; inositol, 400; biotin, 0.6; folacin, 1.5; p-aminobenzoic acid, 40; choline chloride, 800; ascorbic acid, 200; alpha-tocopherol, 40; menadione, 4; beta-carotene, 1.2; activated 7-dehydrocholesterol, 0.0045; and crystalline vitamin B_{12}, 0.009.

The crystalline vitamin supplement, the amino acid supplement, and the alpha-cellulose flour were thoroughly pre-mixed. The complete diet was mixed to contain 25 per cent solids and 75 per cent water, and in mixing, the degree of air incorporation served as a means of providing a diet that floated (for young fish) or slowly sank (for fish as they advanced in age).

Four groups of 1000 yolk-sac chinook salmon fry were placed in screened troughs and taught to feed on finely ground fresh beef liver. After three weeks, when all of the yolk sacs had been absorbed, the fish were feeding actively on the liver diet. Two of the groups were then transferred to the complete vitamin-test diet while the other two groups were continued on the liver diet. Daily mortalities, daily food consumption and bimonthly growth measurements were recorded for a feeding period of 18 weeks.

Those on the test diet increased in average body weight from 0.49 g. to 5.4 g. in 18 weeks while the liver diet groups increased to an average of 6.8 g. Thus while the liver diet seemed somewhat superior to the test diet, the growth, food, and mortality data indicated quite satisfactory performance.

Vitamin deficiencies were studied by deleting from the test diet one water-soluble vitamin at a time in a series of trials with 13,000 chinook salmon fry from three summer-run females. In the vitamin deficiency studies, all fry were fed the test diet without any vitamin supplement for a three-week period in an attempt to decrease body storage. Following the feeding of the depletion diet, specific vitamin deficiencies were studied on groups of 500 fry weighing approximately 1.5 g. each.

As a means of studying recovery from the deficiency, when 20 per cent of the population had died under a given deficiency regimen, the remaining fish were divided into two equal groups. One of these groups was fed the test diet and the other was continued on the deficient diet. Onset of deficiency symptoms varied from a few weeks to ten or 12 weeks, depending upon the vitamin under study.

Thiamin deficiency resulted in poor appetite, muscular atrophy, instability, loss of equilibrium and convulsions prior to death. Riboflavin deficiency exhibited corneal vascularization, cloudy lens, photophobia, incoordination and poor appetite. Pyridoxine deficiency signs were epileptiform fits, hyperirritability, ataxia, anemia, loss of appetite and edema. Pantothenic acid deficiency revealed clubbed gills, prostration, loss of appetite, gills covered with exudate, and general sluggishness.

Inositol deficiency resulted in poor growth, distended stomach, and increased gastric emptying time. Biotin deficiency revealed lesions in the colon, muscle atrophy, fragmentation of erythrocytes, and spastic convulsions. Folic acid deficiency resulted in poor growth, anemia, lethargy and fragility of the caudal fin. Choline deficiency revealed hemorrhages in the kidney and intestine. No

deficiency symptoms could be demonstrated from deletion of either p-aminobenzoic acid or ascorbic acid.

Niacin deficiency was delayed and this was presumed to have been the result of a sparing of this vitamin by the presence of the supplementary tryptophan. A similar delay in choline deficiency development was considered due to the presence of supplementary methionine. Jerky or difficult movement, edema and muscular spasms were observed in niacin deficiency.

Inconclusive results were obtained with respect to the vitamin B_{12}-deficient group, although some fish exhibited poor appetite, erratic hemoglobin and erythrocyte counts, and fragmentation of erythrocytes.

It is evident that the symptoms observed in the salmon parallel rather closely the nature of the deficiency symptoms observed in other species of animals.

When the missing vitamin was returned to the deficient diet, in most cases recovery from deficiency symptoms was rapid. In the case of riboflavin deficiency, where total blindness had occurred, recovery was poor because the fish failed to feed. Recovery with pantothenic acid was only about 50 per cent effective. The opercular lesion in this deficiency appeared to interfere with normal feeding.

In companion studies Halver also investigated the use of amino acid mixtures as a replacement for the protein in the test diet (*J. Nutrition* **62,** *245 (1957)*).

Carboxymethylcellulose was employed as an inert binder for incorporation of the amino acid mixtures into the diets. An L-amino acid mixture, patterned after the amino acids contained in the casein and gelatin of the vitamin test diet, resulted in a 14-week growth response equal to that obtained on the vitamin test diet.

An amino acid mixture, approximating the content of amino acids in yolk-sac fry and fingerling salmon protein, failed to yield acceptable growth responses.

Little is known of the nutrient requirements of fish. It is of interest that in the earlier work with rainbow trout a requirement was demonstrated for pantothenic acid, inositol, and folacin, but niacin, biotin, vitamin B_{12}, tocopherol, and vitamin K deficiencies were not observed (L. E. Wolf, *Prog. Fish Culture* **13,** *17 (1951)*). Improvement in the purity of the test diet, as accomplished in Halver's recent studies, no doubt offers a means of appraising more critically the nutritional needs of fish.

IMPROVING THE NUTRITIONAL VALUE OF BARLEY

The use of barley as a food for man dates back to 5000 B. C. and before. Until the 16th century, barley was the chief grain for breadmaking in Europe and the Mediterranean region, when it was supplanted by wheat and potatoes. Even today barley is one of the world's most widely used grains and can be grown in a greater variety of climates than corn, wheat, or oats. In America, other than its well known use in the brewing industry, barley is used mainly as an animal food. It has been the chief grain in poultry and swine feeds in Canada and in the far West of the United States—outside of the corn and wheat belts.

Barley has always been considered to be definitely inferior in nutritional value in animal feeding as compared with corn or wheat. The reason for this usually has been attributed to a higher fiber content of barley or to poorer digestibility of its carbohydrate. (The proximate analysis of barley shows that its carbohydrate content, aside from crude fiber, is about equal to corn or wheat.)

It is of special interest, therefore, that recent studies from the State College of

Washington show quite convincingly that barley may be made equal to corn in nutritional value for poultry by either treating with water or by feeding enzymes with the barley.

R. E. Fry, J. B. Allred, L. S. Jensen, and J. McGinnis (*Proc. Soc. Exp. Biol. Med.* **95,** *249 (1957)*) describe the effect of water treatment of barley in increasing its nutritional value. Coarsely ground pearled (dehulled) barley was mixed with an equal weight of tap water at 40° C and allowed to stand at room temperature for approximately eight hours. By the end of this time all of the water was absorbed by the grain which was then dried in trays at 70° C for approximately fifteen hours. It was then ground and fed to chicks as part of a practical diet consisting of (in per cent) barley or corn 64.2, soybean oil meal 24.3, fish meal 5, alfalfa meal 2.5, and adequate supplements of minerals and vitamins. Two experiments, consisting of three replicates each and of at least nine chicks per group, were used in comparing corn and barley.

It was found that the water treatment of barley greatly increased its nutritive value, making it equal or superior to corn for poultry. In the two experiments, chicks receiving untreated pearled barley weighed an average of 313 g. at four weeks. Chicks receiving the water-treated barley weighed an average of 404 g. at four weeks, while similar chicks receiving corn instead of barley weighed 373 g. (No description of the corn is given nor do the authors give any results of studies with water-treated corn, unfortunately). Treating the barley with water reduced the amount of feed necessary to make a kilogram of gain from 2.31 to 1.75 kg. This compared with a value of 1.95 kg. for the corn diet.

The authors ascribed the improvement in nutritional value of water-treated barley to an increased availability of energy. It is possible that the water caused a chemical change in the starch granules of the barley resulting in increased digestibility.

This viewpoint is strengthened by another report from this same laboratory which demonstrates that the nutritional value of barley for chicks is significantly improved by feeding it with a mixture of carbohydrate-splitting enzymes (Jensen, Fry, Allred, and McGinnis, *Poultry Sci.* **36,** *919 (1957)*). Procedures similar to those in the previous experiment were used. A mixture of Takadiastase and Clarase (crude carbohydrate-splitting enzymes) was added to the feed containing untreated pearled barley at levels of 0.5, 1.0, and 2.0 g. per pound of diet.

The enzyme supplement, at all levels, significantly improved both growth and feed efficiency as compared with the group fed the barley diet with no enzymes. Chicks receiving the 0.5, 1.0, and 2.0 g. levels of enzymes weighed 356, 375, and 361 g. respectively, while chicks fed the basal ration with no enzymes weighed 319 g. in the three experiments. Control chicks, fed corn in place of barley, weighed 383 g.

The authors suggest that the chick does not produce enzymes which bring about maximum digestion of barley. It appears from these studies that maximum digestibility of barley for the chick can only be obtained by pretreatment of the barley by water or by feeding special enzymes not secreted by the chick.

Although additional studies are needed on the practical application of this finding, of perhaps greater interest to the biochemist would be studies on the mechanism of action of the water or enzymes in improving barley. In any event, it is evident that a definite advance has been made in making barley nutritionally equal to corn for poultry. This finding will undoubtedly be important in areas of the world where barley is the principal grain crop for animal feeding. It is of interest to speculate that similar methods might be used in improving the nutritional value of barley for other monogastric animals, including man. If so, future years may see an upswing in the use of barley as a food.

ORIGIN OF SERUM CHOLESTEROL IN THE RAT

The influence of ingested cholesterol on cholesterol synthesis in the body has been investigated by M. D. Morris *et al.* (*J. Biol. Chem.* **224,** *1039* (*1957*)). Their interest in this subject was aroused by observations that, in man, the level of serum cholesterol remains relatively constant for long periods of time and is not substantially increased during periods in which diets rich in cholesterol are consumed (*Nutrition Reviews* **8,** *341* (*1950*); **13,** *138* (*1955*); **14,** *107* (*1956*)). Although several possible explanations for these observations can be suggested, the possibility that there is a drop in cholesterol synthesis has received support from experiments *in vitro*, in which it has been shown that liver slices prepared from several species of animals previously fed high cholesterol diets show a decreased ability to incorporate isotopically-labeled acetate into cholesterol (*Ibid.* **14,** *85* (*1956*)).

In order to determine, *in vivo*, the relative contributions of dietary and endogenously synthesized cholesterol to serum cholesterol, Morris *et al.* (*loc. cit.*) maintained rats on diets either high or low in cholesterol which had been labeled. The specific activity of serum cholesterol of each group was determined at intervals making it possible to estimate the extent to which the labeled material had been diluted with endogenously synthesized cholesterol.

The rats used for the isotope study were fed *ad libitum* on a purified diet. The diet contained extracted casein, 25 per cent; lard, 11 per cent; glucose, 50 per cent; salt mixture, 6 per cent; and cellulose, 10 or 12 per cent. The diet of one group of rats was supplemented with 0.05 per cent, and that of the other with 2.0 per cent, of cholesterol which had been labeled with C^{14} in the C-4 position of the sterol nucleus. The specific activity of the cholesterol extracted from the diets was identical with that of the cholesterol added. The rats were fed the experimental diets for two to six weeks. At the end of each week three or four animals from each group were anesthetized and blood was removed by heart puncture. The serum was separated, the lipids were extracted, and the cholesterol was isolated as the digitonide. The digitonides were washed, dissolved in methanol and aliquots of the methanol solution were taken for analyses. The cholesterol content of the serum was determined colorimetrically using the ferric chloride method, and the C^{14} content of the cholesterol was determined directly on an aliquot of the digitonide solution.

The results of the experiment were decisive. The specific activity of the serum cholesterol had reached a maximal value in rats receiving the low level of cholesterol within two weeks and remained constant throughout the remaining four weeks of the experiment. The specific activity of the serum cholesterol was from 20 to 33 per cent of that of the dietary cholesterol, indicating that this percentage of the serum cholesterol had been derived from the diet. In contrast, in rats receiving the high level of dietary cholesterol the specific activity of the serum cholesterol did not reach a maximal value until the fourth week. Nevertheless, the values, including the two-week ones, were from 74 to 90 per cent of that of the dietary cholesterol, indicating that of the order of 80 per cent of the serum cholesterol had been derived from the diet. In only one rat of the 40 used in the experiment was the level of serum cholesterol appreciably above the normal range.

The authors indicate that any one of several mechanisms could account for the higher specific activity values of serum cholesterol in rats consuming the higher level of dietary cholesterol. However, in spite of which may be correct of the alternative explanations put forward, the results indicate the relative contributions

of diet and of synthesis to the serum cholesterol. It was not surprising that 70 to 80 per cent of the serum cholesterol should be of synthetic origin when the diet contained only 0.05 per cent of cholesterol. It was, however, unexpected that 26 per cent should be of synthetic origin when the diet contained 2 per cent of cholesterol, particularly since the capacity to incorporate acetate carbon into cholesterol was almost completely suppressed in liver slices from rats fed on high cholesterol diets (*Nutrition Reviews* **14,** *85 (1956)*). This is one more indication of the pitfalls that may be encountered if evidence obtained in experiments *in vitro* is extrapolated to explain observations *in vivo*. It is clear from these experiments that considerable cholesterol synthesis occurs in the intact rat even when a high level of dietary cholesterol is being ingested. It is possible that the liver slice experiments do not indicate accurately the capacity of the liver for synthesis *in vivo* or that synthesis in organs other than the liver contributes substantially to plasma cholesterol.

GROWTH PROMOTING ACTIVITY OF GELATIN

It has long been believed that the nutritional value of proteins lies in their content of amino acids. Yet the amino acids do not support growth nearly as well as protein. The difference is as much as twofold in the case of chicks, less in other species. It is sometimes overlooked because the slower-growing animals eventually reach mature size. Although a number of explanations have been offered, they are inadequate, and further research on the subject continues.

D. A. Benton, H. E. Spivey, and C. A. Elvehjem (*Arch. Biochem. Biophys.* **70,** *491 (1957)*) report on "Properties of substances in gelatin which stimulate the growth of chicks fed amino acid diets." Previous work by this group had demonstrated that chicks fed such diets showed a growth response to proteins, greatest in the case of gelatin and zein, which are both low or lacking in essential amino acids. Because the growth-stimulating activity of gelatin was not destroyed by acid hydrolysis, the authors studied the activity of various fractions of the hydrolyzate.

Groups of ten one-day-old chicks were fed *ad libitum* for two weeks; at four days of age the three chicks with the least growth were removed. The basal diet contained a synthetic mixture of 18 amino acids totalling 23.7 per cent, dextrin (autoclaved cornstarch) 64.9 per cent, soybean oil 5.0 per cent, with suitable salts (6 per cent) and vitamins, including folacin and vitamin B_{12}. Added test fractions were substituted for an equivalent amount of dextrin. Gelatin was hydrolyzed by autoclaving in 6 normal hydrochloric acid, followed by vacuum distillation and neutralization by sodium hydroxide. The neutralized hydrolyzate was treated in various ways alone or in combination: 1) n-butanol extraction (to remove acidic amino acids), 2) phenol extraction, 3) phosphotungstic acid precipitation (to remove basic amino acids), 4) adsorption on the hydroxide form of an ion exchange resin, 5) ashing, 6) adsorption on activated charcoal, 7) lead acetate precipitation, 8) adsorption on the acid form of a resin.

The rate of weight gain of the chicks in one experiment was 2.9 g. per day on the basal diet, increasing linearly with added gelatin up to 5.9 g. per day, when 5 per cent gelatin was added. Various fractions were fed at levels equivalent to 5 per cent of this protein. The growth responses to each fraction were compared with those of a concurrent control, since the rates varied greatly in different experiments. Activities were expressed in percentages of the growth

response to gelatin. The acid hydrolyzate retained most (45 to 107 per cent) of the activity of the gelatin. Although it contained sodium chloride, when 1.5 per cent salt was fed with the basal diet, the growth activity was 20 per cent of that of gelatin. However, some preparations with very little salt had high activity, so that the sodium chloride could be considered at most a contributor to the increase in body weight. Butanol extraction of the hydrolyzate did not remove the activity (7 per cent in the butanol phase, 77 per cent in the water phase). Phenol extraction yielded 10 to 120 per cent activity (six trials) in the phenol phase and 72 per cent (one trial) in the water phase. Charcoal removed the activity.

Because of the great variability in results in different experiments, the authors expressed their conclusions in the most general terms. As they had previously found growth activity in several proteins, they inferred that the active substances might be common protein constituents. Since the hydrolyzate retained growth activity, this could not well be caused by peptides. Since butanol extraction removes most acidic amino acids and phosphotungstic acid precipitates most of the basic ones, and neither one nor both processes removed the activity, amino acids seemed unlikely to harbor the growth activity. The substance was taken up by the resin Dowex 50 (acid cycle), indicating it to be a base, and eluted by 1.5 normal hydrochloric acid, showing it not to be a strong base. The gelatin ash had some growth-stimulating effect (0 to 28 per cent). The authors are pursuing further fractionation and chromatography on Dowex 50 in an effort to define the substance or substances responsible for the growth activity of gelatin.

Many reasons have been offered in the past for the failure of amino acid mixtures (protein hydrolyzates or synthetic mixtures) to give as good growth as protein, but the matter remains unresolved. The biologic completeness of the proteins in these experiments is not in question, and differences in palatability, digestibility and other explanations have not seemed adequate. The matter was somewhat clarified by the discovery of vitamin B_{12} and its congeners. The association of these "animal protein factors" with proteins and their resistance to peptic digestion probably delayed their discovery for a long time. Perhaps similar properties have prevented identification of still other growth-promoting factors in presumably pure proteins.

D. W. Woolley (*J. Biol. Chem.* **159,** *753 (1945)*; *Ibid.* **162,** *383 (1946)*) reported marked stimulation of growth of mice fed casein hydrolyzate when small amounts of casein or trypsinogen were added. Hydrolysis abolished this effect, which paralleled the content of "strepogenin" in various preparations tested. This name had been given to a substance which was an integral part of a number of pure proteins (e.g. casein and trypsinogen) studied by Woolley's group, and which stimulated the growth of certain bacteria. It was liberated by peptic digestion, destroyed by hydrolysis with strong acid or alkali, and proved to be a peptide. Though necessary for growth of some bacteria, strepogenin has not been generally accepted as necessary in mammals.

While one would scarcely expect to find growth-promoting activity in a biologically incomplete, partially degraded protein, such as gelatin, Benton's group has done so. It remains to be seen to what degree they will succeed in explaining why proteins promote growth better than amino acids.

In summary, work is reviewed on growth-promoting activity of gelatin in chicks fed diets containing a synthetic mixture of amino acids. The activity was preserved in hydrolyzates and in certain fractions consistent with the hypothesis that it resides partly or largely in an unidentified weakly basic compound, not an amino acid.

FACTORS AFFECTING IRON ABSORPTION

There is good evidence that ascorbic acid enhances iron absorption. In human studies the absorption of iron in foods was improved by the addition of ascorbic acid or by foods rich in this vitamin (C. V. Moore and R. Dubach, *Tr. Assoc. Am. Physicians* **64,** *245 (1951)*; R. Steinkamp, Dubach and Moore, *Arch. Int. Med.* **95,** *181 (1955)*; *Nutrition Reviews* **13,** *165, 292 (1955)*). The favorable effect of ascorbic acid on iron absorption was also noted in the treatment of nutritional anemia in infants and children, and it was postulated that the vitamin also improved iron utilization by the peripheral tissues (M. K. Gorten and J. E. Bradley, *J. Pediat.* **45,** *1 (1954)*).

In preliminary studies with rats it was observed that there was an interrelationship between ascorbic acid and vitamin E in iron metabolism. Convincing evidence is presented which indicates that a combination of ascorbic acid and vitamin E enhances hemoglobin synthesis and the maintenance of hemoglobin more than does either vitamin alone (S. M. Greenberg, R. G. Tucker, A. E. Heming and J. K. Mathues, *J. Nutrition* **63,** *19 (1957)*).

The experimental animals were raised on an iron-deficient diet from the twelfth day post partum. They were weaned on the twenty-first day after birth and were maintained on an iron-depletion diet until the blood hemoglobin levels were uniformly low (6 g. per 100 ml.).

The basal diet was composed of whole milk powder, 970, casein, 10, and cellulose, 20. The basal diet was fortified with the known accessory factors required by the rat (vitamins and minor elements). Supplements of vitamin E were given orally by dropper twice weekly and supplements of ascorbic acid and iron were given by stomach tube, five days each week. Ferrous sulphate ($FeSO_4 \cdot 7H_2O$, A.R.) was used routinely as the iron source and the vitamin E used consisted of natural mixed tocopherol esters.

At both levels of iron used (2 and 4 mg. per kilogram body weight) hemoglobin regeneration was greater when both vitamin supplements were used than when either was used alone. In seven days the rats receiving both vitamins averaged over 3 g. increase in hemoglobin level while the average was less than 2 g. when either ascorbic acid or vitamin E (7 mg. per rat weekly) alone was administered. In general the more favorable response to the combined vitamin supplement was maintained throughout the three-week experimental period.

Essentially similar results were obtained when the experiment was repeated and the supplement of vitamin E esters was increased to a level of 6 mg. per dose or 12 mg. per week. Unfortunately the level of ascorbic acid supplement used in these experiments was not mentioned.

In another experiment rats were depleted to an average hemoglobin level of 5.3 g. per 100 ml. of blood and then were given three massive doses of iron on the first, sixth and tenth days of the recovery period. Each dose consisted of 25 mg. of Fe per kilogram body weight. The ascorbic acid supplement was given with the iron and vitamin E was also given on the same three days at a level of 12 mg. per dose. An additional dose of vitamin E was given on the forty-sixth day. As in the previously reported experiments, the iron with both vitamin E and ascorbic acid caused the most rapid hemoglobin synthesis and ultimately the highest hemoglobin concentration. Seven days after the last iron dose all rats receiving iron alone or either vitamin with iron, began to decline in hemoglobin concentration while those receiving both vitamins with iron continued to increase hemoglobin synthesis.

Convincing evidence is thus presented to

indicate that iron is converted to hemoglobin more efficiently when given with ascorbic acid and vitamin E. Whether the improvement in efficiency was caused by increased absorption of iron, or by an increased efficiency of utilization in the tissues, could not be determined from these experiments.

Where only three doses of iron were given, it was evident that the animals receiving both vitamins continued to synthesize hemoglobin for a longer period and at an accelerated rate. This could be interpreted as evidence of an improved re-utilization of iron released by the normal turnover of red blood cells. It could also be caused by an influence of the vitamins on iron excretion. A properly balanced oxidation-reduction system, especially in the bone marrow, is no doubt important in determining the rate of transfer of ferric iron, of siderophilin, or of ferritin to the ferrous iron of hemoglobin. A similar mechanism may be operative in the intestine where reduced iron appears to be transformed to the oxidized state in ferritin and then to the reduced (ferrous) state again to cross the cell membrane into the circulation.

That the role of vitamin E in oxidation-reduction systems may be the explanation for its favorable influence in iron utilization was supported by the further studies of these workers where an effective biological antioxidant was substituted for the vitamin E (R. G. Tucker, S. M. Greenberg, A. E. Heming and J. K. Mathues, *J. Nutrition* **63,** *33 (1957)*). So far as is known, the antioxidant used, namely DPPD (N,N′-diphenyl-P-phenylene diamine), is not known to have vitamin E-like biological activity, other than its antioxidant properties. Since it was found to replace effectively the vitamin E as a factor enhancing the utilization of iron for hemoglobin synthesis in the rat, the authors tentatively conclude that the function of vitamin E in the events leading to efficient hemoglobin synthesis probably relate to its role as a biological antioxidant.

It should be pointed out that DPPD was for a time permitted as a poultry feed ingredient because of its strong antioxidant properties, but its use in such feeds has been banned because of toxic effects noted when the compound was used in the diet of pregnant rats (B. L. Oser and M. Oser, *J. Agri. Food Chem.* **4,** *796 (1956)*).

A possible role of vitamin E in hematopoiesis has been observed in several reported studies. I. Indovina noted that vitamin E increased hemoglobin formation in vitamin E-deficient rabbits (*Am. J. Physiol.* **165,** *352 (1951)*). A microcytic anemia in conjunction with a low reticulocyte count has been reported in vitamin E-deficient chicks (M. L. Scott, F. W. Hill, L. C. Norris, D. C. Dobson and T. S. Nelson, *J. Nutrition* **56,** *387 (1955)*). Recently P. L. Day and J. S. Dinning observed anemia in vitamin E-deficient monkeys (*Fed. Proc.* **15,** *548 (1956)*).

A possible interrelationship between vitamin E and ascorbic acid was noted in that the latter appeared to reduce the requirements for the former in the chick (H. Dam, I. Kruse, I. Prange and E. Søndergaard, *Biochim. et biophys. acta* **2,** *501 (1948)*).

Whether or not the influence of vitamin E on iron utilization for hemoglobin synthesis is mediated through more efficient iron absorption, or more efficient utilization at the tissue level, remains to be determined. The authors recognize that studies with radioactive iron would help to explain some of their observations.

THE NUTRITIVE VALUE OF BACTERIAL PROTEINS

The biological value of bacterial protein is of interest because it is known to represent, to a considerable extent, the protein available to ruminants. Rumen microorganisms after they leave the rumen, are digested in the true stomach and the small intestine of ruminants. The mixed microbial population includes many forms which are able to convert nonprotein nitrogen into bacterial protein during fermentation in the rumen. Since many of these bacteria also degrade and convert feed protein into bacterial protein, it is of importance to establish the value of the bacterial protein.

A recent study at the University of Illinois reports fundamental differences in biological value and in digestibility between two nutritionally distinct bacterial types (B. Kaufman, W. O. Nelson, R. E. Brown, and R. M. Forbes, *J. Dairy Sci.* **40,** *847 (1957)*). *Escherichia coli* was selected to represent an organism nutritionally independent in regard to pre-formed amino acids, while *Lactobacillus arabinosis* was selected to serve as the bacterial source which is dependent upon organic nitrogen for growth. *E. coli* was grown on a synthetic ammonium sulphate medium and also on a complex tryptone-containing medium. *L. arabinosis* was produced on the organic medium.

The harvested (centrifuged and washed) bacterial cells served as the dietary protein source in biological evaluation and digestibility studies in rats. The *E. coli* cells were fed untreated, (live) lyophylized, or, sonic-oscillated and lyophilized. *L. arabinosis* cells were fed untreated or lyophilized.

The biological value, defined as the percentage of digested protein that was retained, was determined by feeding the bacterial cells preparations in a purified diet at a level to provide 12 per cent of protein (N x 6.25). Endogenous urinary and metabolic fecal nitrogen values were calculated from data obtained during a preliminary period when the purified diet (24 parts) and hexane-extracted whole egg (1 part) were fed.

The digestibility of *E. coli* protein (grown on synthetic medium) was found to range between 80 and 85 per cent, with the dried cells exhibiting the higher digestibility. Biological values ranged between 67 and 70 per cent, with no significant difference between untreated (living) or dried *E. coli* cell protein. Casein was included for comparison, and both the digestibility (99 per cent) and the biological value (82 per cent) of this protein were significantly higher than the values for *E. coli* protein.

With *L. arabinosis* cell protein digestibilities ranged between 84 and 89 per cent whereas biological values of 52 to 56 per cent were found. The dried cells, again, were somewhat better than the untreated cells.

From these data it is evident that the protein in *L. arabinosis* is more digestible than that in *E. coli*, but the reverse is true with regard to their biological values.

A direct comparison of *E. coli* and *L. arabinosis* cells, both grown on tryptone medium, yielded a digestibility and a biological value of 86 and 61, respectively, for *E. coli*, compared with 90 and 48, respectively, for *L. arabinosis*. The similar behavior of *E. coli*, whether grown on synthetic or organic media, substantiated the hypothesis that the biological value of bacterial protein is a function of species or genus, rather than a function of the medium on which the cells are grown. When *E. coli* was grown on the two media, and fed in a direct comparison, the digestibilities and biological values were 86 and 61, respectively.

The low biological value of the protein of *E. coli* could be improved significantly by the addition of five amino acids (histidine, lysine, methionine, phenylalanine, and tryptophan). The single addition of methi-

onine was as effective as the five amino acids, although none of the amino acid additions improved the biological value to equal that of casein.

Contrasted with the low biological values obtained for bacterial proteins in the present study, two groups of workers have reported biological values equal to, or above, casein in studies where mixed rumen bacterial cells served as protein source (M. L. McNaught, J. A. B. Smith, K. M. Henry, and S. D. Kon, *Biochem J.* **46,** *32 (1950)*; F. M. Reed, R. J. Moir, and E. J. Underwood, *Austral. J. Sci. Res.* **2,** *304 (1949)*). The mixed bacterial populations used by these workers probably would provide a better amino acid balance and thus result in a higher biological value.

Two reports have appeared which suggest that mixed rumen bacterial populations vary in their amino acid composition, depending upon the ration fed. Rumen bacteria in sheep fed green forage contained more methionine than those in sheep fed dry rations (*Reed, et al., loc. cit.*). Tryptophan was found to be a limiting amino acid in the protein of bacteria found in sheep that were fed casein or gelatin (W. C. Ellis, G. B. Garner, M. E. Muhrer, and W. H. Pfander, *J. Animal Sci.* **14,** *1200 (1955)*). Based upon the careful Illinois studies, these variations in amino acid composition of mixed rumen bacterial populations probably represent species shifts within the flora rather than changes in amino acid composition of individual species.

Since it has been difficult, or impossible, to demonstrate any benefit from amino acid supplementation of the ration in ruminants, regardless of quality of protein used in the ration, there is some question as to the usefulness of the rat as a test animal in evaluating the protein quality of ruminant rations or their fermentation products. It is possible that the ruminant is particularly adapted to the protein derived from bacterial fermentation. Perhaps the young calf, in which rumen fermentation is dormant, should serve as the test animal in determining the biological value of bacterial proteins.

ESSENTIAL FATTY ACIDS IN CELL PERMEABILITY

It has been known for some time that certain unsaturated fatty acids may influence the growth of many gram-positive bacteria, both negatively and positively (C. Nieman, *Bacteriological Reviews* **18,** *147 (1954)*). The reasons for these actions have, however, remained obscure and controversial. Two possible explanations for the growth promoting effect have been advanced: 1.) biotin functions in the biosynthesis of these nutritionally essential fatty acids (W. L. Williams, H. P. Broquist and E. E. Snell, *J. Biol. Chem.* **170,** *619 (1947)*); or 2.) both biotin and the fatty acids somehow function in cell permeability (V. R. Williams and E. A. Fieger, *J. Biol. Chem.* **170,** *399 (1947)*). The first of these possibilities seems unlikely because of the diverse nature of these essential fatty acids and the lack of any real evidence for this type of action by biotin. The second has not been entirely satisfactory because of the great discrepancy in the amounts of biotin and fatty acid necessary (over 10,000 times as much of the latter).

A possible resolution of this problem has been suggested by A. Traub and H. C. Lichstein (*Arch. Biochem. Biophys.* **62,** *222 (1956)*).

The authors started with the working hypothesis that oleic acid functions only in promoting cell permeability to biotin. Initial findings were that *Lactobacillus arabinosus* cultures grew more rapidly at pH 6, or at pH 6.8 in the presence of Tween 40, than at pH 6.8 in its absence. Moreover, some growth was observed at pH 6 (but not at pH 6.8) even in the absence of added

biotin. Thus it was thought that the medium used must contain small amounts of biotin which could be utilized more efficiently at the lower pH, or in the presence of emulsifying agent. As confirmation of this hypothesis, it was found that added malate also stimulated growth on the low-biotin medium, but not when the last traces of biotin were removed, by treatment with Norit, from the commercial hydrolyzed casein employed in the medium. Thus malate could stimulate growth only in the presence of at least traces of biotin.

That this reasoning also applies to growth stimulation by oleic acid was also shown. With suboptimal levels of biotin, the same conditions (as lower pH) necessary for utilization of biotin proved necessary for the oleic acid effect. Moreover, when the last traces of biotin were removed from the medium by treatment of the Casamino acids with Norit, oleic acid had no effect on growth. Thus the evidence appears strong that the function of oleic acid and the other growth promoting fatty acids and surface-active agents is in promoting the passage of certain substances, such as biotin, across the cell membrane.

In a further study with the same organism, Traub and Lichstein found that the growth promoting effect of malate is due largely to its ability to enhance the formation of carbon dioxide (which has growth stimulating properties for Lactobacilli), probably through adaptive formation of the malic enzyme system (malate + $TPN^+ \rightleftharpoons$ pyruvate + carbon dioxide + TPNH). It was interesting that the unsaturated acids, (oleic, linoleic and linolenic) but not the saturated fatty acids (palmitic and stearic) caused an inhibition of the carbon dioxide formation in the presence of malate. This effect was apparent with intact cells, but not with a cell-free preparation. In this case, the authors reasoned, either too great a permeability to an essential intracellular substance or, as seems more likely, too low a permeability to the water-soluble malate, was caused by the added fatty acids.

From the results of these experiments, it appears that many of the growth promoting, as well as the growth inhibiting, properties of unsaturated fatty acids for microorganisms can be explained logically. One of the several functions of the essential fatty acids in higher organisms may be of a similar nature.

LONG TERM INGESTION OF FLUORIDES

There has been considerable discussion, especially during the past decade, as to the safety of consuming fluoridated water over long periods of time. There is, however, no question of the beneficial dental effects resulting from the ingestion of water containing small amounts of fluoride during tooth development (*Nutrition Reviews* **11,** *324 (1953)*). A low incidence of dental caries occurs whether the water naturally contains approximately 1 p.p.m. of fluoride or has been supplemented to contain that amount.

For some time, considerable public health effort has been expended to increase the number of people who might benefit from the consumption of fluoridated water. One out of four people living in urban areas of the United States now are using water "with an adjusted fluoride content" (*Div. Dental Public Health, Public Health Service, Public Reports* **71,** *963 (1956)*). The expansion of this public health measure has met with a certain amount of opposition. In the United States, as of January 1955, the fluoridation of public water supplies was submitted to a vote in 194 communities. The proposal was defeated in 108 communities and approved in 86 (N. C. Leone, *Texas State J. Med.* **51,** *457 (1955)*).

The public discussion of the merits of fluoridation has evoked much emotionalism that is based largely on fears of potential toxicity which may result from the prolonged ingestion of low levels of fluorides.

Considerable data are available on the acute toxicity of fluorides and there is an accumulating amount of data on the effect of using water with different levels of fluorides. Much of the latter information pertains to human beings.

One study that attempted to provide the above type of data for rats was that of W. F. Ramseyer, C. A. H. Smith and C. M. McCay (*J. Gerontology* **12,** *14 (1957)*). They gave weanling rats distilled water to which was added either 0, 1, 5 or 10 p.p.m. of fluorine. The rats were fed a ration containing in per cent: toasted wheat flakes, 65; skim milk powder, 10; soybean meal, 10; brewers' yeast, 6; alfalfa leaf meal, 5; corn oil, 2; salts, 1; and liver meal, 1. A single analysis of this diet showed it to contain 14 p.p.m. of fluorine, which is an unusually high value for such a diet. Another batch of the same diet prepared three years later had so little fluorine that it "was too low to measure." The latter statement is difficult to comprehend unless a very insensitive method for fluoride was used, as a diet of the above composition will routinely contain 0.5 to 2.0 p.p.m. of fluorides.

Forty-eight pregnant rats were divided into four groups. All received the above diet and water containing one of the fluoride levels. The offspring of these animals (165 rats) were maintained on the regimen for 150 days, when one-half the animals in each group was killed; the remaining animals were kept on the experiment for 520 days, when they were killed.

None of the animals killed at the end of 150 days showed any caries. Of the rats maintained for 520 days, the 21 receiving no fluorine in the water had no caries. In the other groups there was a total of 3, 13, and 9 carious molar teeth in the 17, 24, and 24 rats receiving 1, 5 and 10 p.p.m. of fluorine, respectively. The number of missing teeth in the various groups was stated to be the same as the number of carious teeth. Presumably the authors assumed that a missing tooth had been destroyed by caries without consideration of possible losses due to trauma or peridontal disease. All teeth were present in the 520-day-old rats receiving no fluorine in the water. The other groups had three or four rats with one to six teeth missing.

It was stated that there was no sign of peridontal disease in the rats sacrificed at 150 days. Of the rats killed at 520 days, only two were reported to have peridontal disease in the group receiving no fluoride in the drinking water. The investigators indicated there were signs of peridontal disease in all of the older animals receiving sodium fluoride added to the drinking water. According to their statement, this was evident by the alveolar bone destruction and increased porosity of the bone. No evaluation of the extent of peridontal changes was reported, nor was the variation from group to group of those rats consuming different levels of fluoride.

The addition of sodium fluoride to the drinking water had no effect on the density of the femurs of the rats killed at either 150 or 520 days. The fluoride concentration of the bones was proportional to the fluoride in the drinking water. The data indicate that the female rats stored more fluorine in the bones than the males. This was especially true for the groups receiving the higher levels of fluoride intake. It is significant that, for the rats receiving the lower fluoride intakes (0 and 1 p.p.m. in the water), there was no increase in the concentration of the mineral in the femur when the results for the 150-day-old rats were compared with those for the 520-day-old animals. For the rats on the higher intakes, the standard deviations were so large that it makes evaluation of the data very difficult. The investigators presented practically no statistical information or evaluation of their data.

No macroscopic or microscopic changes were apparent in the thyroids of any of the rats. No macroscopic changes were seen in the kidneys of any rats. Microscopic examination showed interstitial nephritis, the severity of which was reported to be pro-

portional to the level of fluoride in the drinking water. Tubule hypertrophy and hyperplasia occurred in the animals receiving fluoride in the water but not in those animals receiving no fluoride in the water. Unfortunately, the kidneys from only a small and variable number of rats (6 on 0; 3 on 1; 1 on 5; and 6 on 10 p.p.m. of fluoride) were examined microscopically. No information was presented to indicate why these particular rats were chosen for the histopathologic examination.

The interpretation of the report by Ramseyer, Smith and McCay (*loc. cit.*) is very difficult due to a number of inherent weaknesses, especially in regard to the design of the experiment. In the first place, the investigators stressed the fact that there was considerable variation among animals. Since all the offspring of a pregnant rat were placed on the same fluoride intake, there is a possibility that genetic differences might be responsible for the dental caries and kidney lesions observed in the older rats. It is amazing in an era when so many clearly demonstrated evidences of genetic relationships to dental diseases are available that any investigator would fail to distribute litters uniformly among experimental and control groups. The litter sizes must have been very small (average = 3.4) since there were only 165 offspring from 48 pregnant rats. At the critical period of observation (520 days) when all of the reported complications resulting from the ingestion of fluoridated water occurred, there were only 22, 16, 19 and 20 rats in the four groups.

The investigators (Ramseyer, Smith and McCay, *loc. cit.*) carried out a similar study in which a larger group of rats was maintained for 150 days. Again, at the end of 150 days, very few group differences were apparent. It is hard to see why the experiment was terminated at that stage when the reported effects of fluoride on the kidneys and teeth in the previous experiment were seen only after 520 days.

The extent and degree of the peridontal disease cannot be evaluated from the meager statements given by Ramseyer, Smith and McCay (*loc. cit.*). It is safe to assume that the presence of increased amounts of fluoride in the drinking water has not been responsible for the increased incidence of dental caries in this investigation. All other data from a variety of laboratories show that in the rat, as in humans, dental caries can be reduced by this means (*Nutrition Reviews* **13,** *30 (1955)*). However, in the rat, it has been clearly demonstrated that between 15 and 20 p.p.m. of fluoride is necessary to cause a reduction in caries incidence. The highest level used by Ramseyer *et al.* falls far below this level. The difference in caries incidence in these groups probably reflects the lack of littermate distribution in a strain that is highly variable with respect to the individual rat's susceptibility to dental caries.

The data on fluorine intake are not as complete as one might desire for such a study. It is stated that one batch of the diet on analysis contained 14 p.p.m. of fluorine which is considerably higher than would be expected. Another batch of the same diet prepared three years later had an undetectable amount. Since the volume of water consumed is likely to be about the same as the weight of food ingested, this would make the diet used over most of the experimental period an important contributor to the overall fluorine intake. For that reason, the diet should have been analyzed more frequently. In all probability, food and water intakes by individual rats should also have been determined if data capable of thorough interpretation were desired.

The public health importance of any study dealing with the long term ingestion of fluoride requires a careful consideration of all data bearing on the problem before any decision is made. For this reason, it is advisable to note the results secured by R. L. Maurer and H. G. Day (*J. Nutrition* **62,** *561 (1957)*). They fed rats a purified ration, that was very low in fluorine (0.6 p.p.m.), for three generations. Control rats receiving the same ration were given 2 p.p.m. of fluoride (as sodium fluoride) in the drinking

water. There was no indication of any accumulation of fluoride in the rats born in the successive generations, since all the offspring analyzed were essentially fluoride-free. Even after the rats had been on the experiment for 325 days, there were no caries or other dental defects in either group that were detectable by means of a dental probe. Furthermore, in so far as phosphatase activity is a measure of kidney function, the ingestion of fluoride for 325 days had no effect on this enzyme level.

Another long term study of fluorine intake was made by A. W. Pierce (*Australian J. Agr. Res.* **3,** *326 (1952)*), who placed eleven-month-old sheep on a ration of hay, wheat and linseed meal. One group of five sheep received rain water containing no fluoride; other groups of five received rain water to which 2.5, 5, 10 and 20 p.p.m. of fluoride had been added. Over a period of three and a half years, the growth and general appearance of the sheep were the same whether they received water or water containing 2.5 p.p.m. of fluoride. The higher levels of fluoride in the water (5, 10 and 20 p.p.m.) produced a slight reduction in growth, especially after the second year on the experiment. There was, however, no consistent difference in water consumption by any of the groups. Had there been any serious disturbance in kidney function resulting from these levels of fluoride intake, some alteration in water consumption should have been apparent.

It should be pointed out that there are reports of kidney disturbances associated with severe fluorosis. When rats were fed rations containing from 210 to 700 p.p.m. of fluoride, the kidneys showed "parenchymatous degeneration in the convoluted tubules, and occasionally hyaline degeneration in the blood vessels" (P. H. Phillips and A. R. Lamb, *Arch. Pathol.* **17,** *169 (1934)*). In these experiments, the kidneys and thyroids were the soft tissues that most frequently showed abnormalities.

H. E. Shortt, *et al.*, (*Indian J. Med. Res.* **25,** *553 (1937)*) found abnormalities in the filtration rate and blood urea clearance of six of ten patients who had been hospitalized because their fluorosis was so severe that they could hardly move. The urinary fluoride levels (1.58 to 4.08 mg. per cent) and the severe mottling of the teeth suggest that the intake of this substance must have been very high. Results somewhat similar to these have been reported by A. H. Siddiqui (*Brit. Med. J.* **II,** *1409 (1955)*) for 32 cases of severe fluorosis from another part of India. The kidney damage observed in both of these groups may be partially due to the severe malnutrition seen in both areas from which the patients came. The findings do suggest that other tissues such as bones and nerves are disturbed earlier and to a greater extent than the kidneys (resulting from the pressure exerted on them by exostoses, probably central in origin).

With lower levels of fluoride in the water, no signs of kidney disturbance have been reported. A survey of the residents of Bartlett, Texas, where the water supply had 8 p.p.m. of fluoride, showed no greater signs of kidney disturbance than in neighboring Cameron where there was 0.4 p.p.m. of fluoride in the water (Leone, *et al., Fluoridation as a Public Health Measure, Am. Assoc. for Advancement of Science, Washington, D. C. (1954)*). The condition of the kidneys was based on the absence or presence of albumin in the urine. Each subject for the study had been a resident in the community for 15 consecutive years prior to 1943. At that time a medical examination was given 116 residents in Bartlett and 121 in Cameron. In 1953, 96 of the original subjects of Bartlett, and 113 in Cameron, were re-examined (Leone, *et al., Public Health Reports* **69,** *925 (1954)*). Except for the high incidence of dental fluorosis (mottled enamel) among the residents of Bartlett, there was no difference in the health or medical histories of the people in the two communities.

Similar results were noted in the New-

burgh-Kingston study (*Nutrition Reviews* **9,** *21 (1951)*), where, after eight years of water fluoridation, the incidence of kidney damage was the same in the two cities (E. R. Schlesinger, D. E. Overton, and H. C. Chase, *J. Am. Med. Assn.* **160,** *21 (1956)*). Overnight urine samples secured from 100 twelve year-old boys in each of the cities showed essentially the same distribution of values for albumin, red cells, casts and specific gravities. If there were any differences, they "tended to favor the Newburgh children" where the water contained 1.2 p.p.m. of fluoride.

F. J. McClure (*Nutrition Reviews* **11,** *326 (1953)*) reported that the mortality rates from nephritis from the period 1945 to 1949 were the same for Stevens Point, where the water contained 0.2 p.p.m., and Green Bay, Wisconsin, where the water naturally contained 2.5 p.p.m. There was a decreased mortality from nephritis in Sheboygan, Wisconsin in the five-year period after the initiation of the fluoridation program compared to the five prior years.

These studies come at a time when additional factual information on the long term effects of fluoride ingestion is needed. The work of Ramseyer, Smith, and McCay (*loc. cit.*) should not be interpreted as evidence condemning the use of fluoridated water for the control of dental caries. On the other hand, it should be recognized that some long term studies on the effects of fluoride should be carried out where careful precautions are taken to control the amount of fluoride consumed both in the ration and in the drinking water supply and to control the genetic composition of the groups through littermate distribution.

EXPERIMENTAL LIPOGENESIS

The study of the mechanism by which fat is deposited in cells is of potential significance in developing an understanding of the nature of fatty changes in many tissues, including atherosclerosis of arteries. D. G. Cogan and T. Kuwabara (*Arch. Path.* **63,** *381 (1957)*) have developed a method for studying what they term "aberrant lipogenesis." This method involves the investigation of the factors which influence the deposition of fat in cells which are incubated in the presence of sodium oleate or other substances containing oleate.

Because of its uniform cellular structure and a normal lack of blood vessels the cornea was used in the early studies being reported, but later experiments reveal that lipogenesis occurs in the experimental system in other tissues as well. The technique of study consisted in cutting rabbit corneas into pieces and incubating them under sterile conditions in the test solution at 37°C. for 24 to 48 hours. One mg. of sodium oleate in 0.01 ml. of solution was used per ml. of serum medium.

At the end of the experiment the pieces were fixed in 5 per cent neutral formalin, and frozen sections were made and stained either with Oil Red O or Sudan IV. Located first around the nucleus, the intracellular sudanophilic globules appeared in six hours, and within 24 to 48 hours they occupied a large part of the cytoplasm. All cells were involved but the globules were most evident in the epithelium. The optimum pH ranged from 6.8 to 8.0. The discovery that serum was necessary for the implementation of this form of lipogenesis led to this present report, which gives the conditions investigated in an effort to define the nature of the serum factor involved.

The species from which serum or cornea was taken did not affect the lipogenesis which occurred in either rabbit, guinea pig or human cornea with serum from rabbit, guinea pig, horse or human sources. Dilution of serum, either with 0.9 per cent sodium chloride or Tyrode's solution, resulted in decreased lipogenesis. The possibility that serum acted as a detoxifying agent for

oleates was eliminated when it was found that dilution of both serum and oleate also resulted in a lessening of lipogenesis. Thus the reduction in serum was the cause of the decrease in lipogenesis.

Moderate heating of serum to 60°C. did not alter its lipogenic ability but heating to 100°C., with almost complete coagulation of protein, resulted in a nearly complete loss of lipogenic potency. Refrigeration at 5°C., or freezing at −80°C., did not reduce the lipogenic process. Vacuum drying of the serum, either at room temperature or at freezing temperatures, followed by reconstitution, did not effect a reduction in the lipogenic phenomenon. Serum which was refrigerated for a period of two to three weeks or incubated for two to seven days showed no evidence of loss of the lipogenic agent. Extraction of ether soluble blood lipids did not remove the factor promoting lipogenesis. Dialysis of serum eliminated the ability to promote lipogenesis by either the dialysate or residue, but remixing dialysate with residue effectively restored the ability, indicating that two components were necessary.

Addition of numerous serum protein fractions to Tyrode's solution, which is roughly equivalent to the non-protein part of serum, failed to reveal any single protein as the responsible agent. The proteins used included crystallized albumin, albumin-oleic acid, ammonium sulfate fractionated albumin globulin or albumin-globulin, Cohn's serum factors I through V, serum mucoprotein or lipoprotein. Thus the essential protein may have been rendered ineffective during preparation or may be a multiple agent. The addition of Tyrode's solution in place of the dialysate of serum returned the lipogenic activity to normal, the responsible substances being calcium and, to some extent, magnesium chloride. Lipogenesis did not occur in cornea which was incubated in sodium chloride, dextrose, glycerin, Tyrode's solution, phosphate buffer (pH 7.49), hemoglobin, aqueous humor, polysorbate 80 in Tyrode's solution, Simm's lipfanogen, or macerated extracts of liver, heart, kidney and spleen suspended in Tyrode's solution.

Kuwabara and Cogan (*Arch. Path.* **63**, *496 (1957)*) have evaluated the extent of the specificity of oleates as a substrate and they have considered the possible correlation of the oleate induced condition with the origin of fat in fatty degeneration. Injection of oleic acid into the corneas of living rabbits resulted in progressive inflammation and necrosis. Histological examination at intervals showed that fat droplets appeared as early as 12 hours and continued to increase in amount over several days. Finally the invasion of inflammatory cells masked the reaction. Similar results were obtained using sodium oleate (adjusted to pH 7.2). When either sodium oleate or oleic acid was injected into excised corneas, which were then cut in pieces and incubated in serum, the cells near the injection site disappeared and fat was found in the adjacent cells starting at six hours and continuing for at least seven days. The fat formation from sodium oleate was more extensive while that from oleic acid was limited to the area near the necrotic tissue. Calcium oleate injected into the corneas of living rabbits produced a less marked lipogenesis than sodium oleate, but also caused less inflammation. Methyl oleate elicited a response identical with sodium oleate.

Tween 80, the oleate ester of sorbitol, when injected into corneas or incorporated in the serum incubation medium at an optimal concentration of 10 mg. per ml., produced the same extensive necrosis and lipid staining obtained using sodium oleate. Again, however, no lipogenesis was obtained in the absence of serum proteins. Equivalent concentrations of non-oleates including the organic acids acetic, butyric, n-caproic, undecylenic, stearic, palmitic, and arachidic, produced no lipogenesis. Sodium acetate, butyrate, purified elaidate, stearate or palmitate were ineffective but sodium linoleate of questionable purity yielded lipogenesis. Also ineffective were glycerol monostearate, methyl stearate, ethyl linoleate, polyoxyethylene sorbitan monostearate

(Tween 60), polyoxyethylene sorbitan monopalmitate (Tween 40) and polyoxyethylene sorbitan monolaurate (Tween 20). Thus oleic acid and its soaps or esters alone appears capable of producing the type of lipogenesis noted in these experiments.

Fat extracted from rabbit omentum and injected into rabbit cornea was detectable for a long time but did not produce the intracellular globules seen usually. Similarly neutral fat or cholesterol injected in pieces of cornea, or put into the serum medium, did not stimulate lipogenesis. Injection of olive oil, triolein, or paraffin into corneas of living animals was ineffective unless first saponified. In contrast, the oleate of a phospholipid was very active in lipogenesis without saponification. Whole rabbit blood or washed red cells injected into living rabbit corneas resulted in fat formation but it did not appear until the second week and was not accompanied by necrosis or inflammation. Rabbit plasma injected into rabbit cornea gave rise to considerable fat. The use of serum from patients with xanthomatoses did not augment this effect.

Fat formation was seen occasionally in corneal pieces incubated in serum with no addition of oleates. This did not occur if lipids were first removed from the serum, but increased if the donor animals were heparinized within an hour prior to drawing the blood. One patient who had xanthomatosis and had received heparin one hour prior to drawing blood yielded a serum sample which promoted the greatest amount of fat formation. Heparin added *in vitro* does not have this effect. When plasma of cholesterol fed rabbits was injected, the masking effect of large amounts of free fat made it difficult to study new fat formation.

As a test to learn whether corneal damage could be responsible for lipogenesis, a sample of corneal tissue was ground with sand, resuspended and injected. Only trace amounts of fat formation resulted and only occasionally. Emulsified whole liver, the ether extract of whole liver, and the residue after ether extraction all induced an active lipogenesis if injected into corneas *in vitro* but not when they were added to the serum incubation medium. Further extraction of liver with methanol and chloroform abolished the residual lipogenic capability. The lack of necrosis or inflammation appeared to be related to the binding of oleates by other tissue constituents. Similarly prepared emulsions or extracts of brain produced identical effects.

Cogan and Kuwabara (*Arch. Path.* **64,** *23 (1957)*) continued their investigation of lipogenesis by directing their attention toward the processes involved within the cell, and secondly toward comparison of lipogenic activity in different tissues. In the cornea all cell types were capable of showing lipogenesis to approximately the same extent with the substrate dissolved in the medium. The basal cells of the epithelium showed it earlier and more intensely than the more superficial epithelial cells. Injection of oleate into the cornea initiated lipogenesis more markedly in the surviving adjacent cells. Epithelial or endothelial surface cells showed lipogenesis after the stromal cells had disappeared.

Although there was no apparent difference in lipogenesis in the central or peripheral portions of the cornea, blood vessels were more resistant than adjacent stromal cells. Epithelium or stromal cells showed lipogenesis even when separated, indicating that the whole tissue was not essential to the process. Although the fat globules formed first about the nucleus, they remained exclusively in the cytoplasm. No lipase activity was found either histochemically or by measuring titratable acid. No difference in the extent of the process was seen in corneas from human, beef, cat, rat, guinea pig or rabbit sources. Fat appeared first in six hours and increased in amount until five days. Although the globules increased in size they tended to remain as distinct globules. Incubation of corneas at various pH ranges indicated that activity was limited to the area of pH 6 to 8. Although some lipogenesis occurred at room tempera-

ture (20 to 24°C.), 37°C. was optimal and extremes of 4 to 5°C. and 55 to 60°C. showed no activity. Complete inhibition of lipogenesis resulted from heating to 60°C. for five minutes, but addition of 0.0025 M potassium cyanide or dinitrophenol caused only partial inhibition, as did incubation in an atmosphere of nitrogen.

Storage of corneas at 2 to 5°C. for five days did not reduce the lipogenic capability but longer storage reduced the potentiality, which disappeared after 11 days or longer. Soaking of pieces of cornea in distilled water one half hour before incubation eliminated lipogenesis completely but no loss was observed by soaking in isotonic saline for 18 hours, Tyrode's solution for four days, or chicken embryo extract for two weeks. Irradiation with 15,000 rep. of strontium beta emission did not reduce lipogenesis whether incubation followed immediately or one month later. Mechanical trauma resulting from pressure, however, was enough to cause disappearance of injured cells following incubation. These findings suggested that the phenomenon was an intracellular process enzymatically catalyzed, metal dependent and requiring energy from phosphorylating reactions.

Liver was also chosen for study since in contrast to cornea which is fibrous it consists chiefly of parenchymatous tissue and has a significant role in lipid metabolism. Liver cells differ from corneal cells in that they contain fat, normally, and also glycogen. As a result the substrate was present, constantly permitting lipogenesis to occur with no addition of oleates. One other outstanding difference was the appearance of lipogenesis in surviving marginal cells, suggesting the necessity for a proximity to oxygen or essential serum factors. The cord cells of the liver, Kuppfer cells, bile duct epithelium and portal veins all showed lipogenesis in the marginal zones, the phenomenon occurring in guinea pig, rat, and rabbit samples. In contrast to cornea, liver showed an earlier appearance of lipogenesis, which was maximal in 24 hours.

Abolition of lipogenesis occurred when M/80 KCN was added to the medium, but the use of M/400 dinitrophenol merely diminished the extent. When fat forms in the liver adjacent to an area of necrosis these authors call the process "fatty degeneration." A comparable process was demonstrated in liver tissue made necrotic by *in vivo* freezing, followed by later removal. The release of oleate from the necrotic cells may have served as a substrate for lipogenesis in the nearby normal cells.

The study of lipogenesis in numerous tissues revealed a response similar either to cornea or liver and with a great variation in capacity. Aorta, connective tissue (including scar tissue), sclera, tracheal, nasal and tongue mucosa, umbilical stroma, hypophysis, and probably heart showed the typical corneal type of reaction, while kidney, lung, granulation tissue, ovary, choroid plexus of the brain, some muscle, parathyroid gland and spleen exhibited the marginal, oleate-independent form of lipogenesis. Lipogenesis was not found in adipose tissue, intestinal mucosa, nerve tissue, lymph nodes, polymorphonuclear leukocytes, thyroid gland and some muscles.

The experiments are interpreted to mean that "fatty degeneration" and atherogenesis result from enzymatic synthesis of fat rather than from the unmasking or phagocytosis of preformed fat. Although Cogan and Kuwabara observed an abundant lipogenesis in cells of the media of the aorta, usually an atheroma develops in the intima. Since it was impossible to obtain human preparations which were fresh enough to test for lipogenesis, future experiments were suggested in which intima undergoing fibroblastic proliferation would be used to see whether lipogenesis could occur. Another projected experiment would endeavor to characterize the fat formed. This is necessary if one is to demonstrate, with certainty, that the cells are forming the lipid rather than removing preformed material from adjacent tissue and fluids.

INOSITOL REQUIREMENT OF TISSUES

The status of myo-inositol as a member of the vitamin B complex has been the subject of considerable controversy. This compound is known to be a growth factor for a number of yeasts and molds. Also, symptoms which respond to the administration of myo-inositol have been observed in several species of animals, particularly in mice (*Nutrition Reviews* **2,** *163 (1944)*; **3,** *88 (1945)*; **4,** *163 (1946)*). It was established early that the rat could excrete more inositol than it consumed (Needham, *Biochem. J.* **18,** *891 (1924)*), and recent work has shown that the young rat can synthesize inositol from glucose (*Nutrition Reviews* **14,** *219 (1956)*). The actual site of the synthesis has not been established, however, and the relative contributions of the tissues and of the intestinal microflora remain in doubt. It is, therefore, of considerable interest that H. Eagle, V. I. Oyama, M. Levy and A. E. Freeman (*J. Biol. Chem.* **226,** *191 (1957)*) have been able to demonstrate a requirement for myo-inositol by several human cell lines grown in tissue culture.

The development of a well defined medium which supported the growth of isolated mammalian cells was a prerequisite to the study of specific tissue requirements (*Nutrition Reviews* **13,** *344 (1955)*; **14,** *89 (1956)*). The basal medium used in the current study contained only seven vitamins (choline, folic acid, nicotinamide, pantothenate, pyridoxal, riboflavin and thiamine), thirteen essential amino acids, glucose and a salt mixture, together with a small amount of dialyzed serum. This medium had previously been shown to support the growth of a human malignant cell and a mouse fibroblast (*loc. cit.*). However, when a number of other normal and malignant cell lines were cultured in this medium only one of 18 could be maintained in a satisfactory state for more than a few days. With the addition of a small amount of serum ultrafiltrate to the basal medium all of these cell lines survived and grew at a satisfactory rate.

These observations served as the starting point for subsequent studies on the growth factor requirements of the cell lines that did not multiply rapidly unless they were supplied with serum ultrafiltrate. Eagle *et al.* tested some 90 compounds for their growth promoting activity and for their ability to replace the serum ultrafiltrate. Of the compounds tested only myo-inositol could replace the serum ultrafiltrate; none of the other compounds, alone or in combination, had demonstrable activity. Also, every one of the cell lines tested proved capable of sustained growth in the medium supplemented with myo-inositol. With further refinement of the medium it was possible to demonstrate an inositol requirement for the malignant cell line previously grown without additional inositol, leaving the mouse fibroblast as the only cell for which no inositol requirement could be demonstrated.

Seven of the eight isomers of myo-inositol were tested for their growth promoting activity but none proved effective. A group of structurally related compounds likewise proved ineffective in replacing myo-inositol. Only phytic acid and two preparations of inositol monophosphate (one from enzymatic hydrolysis of phytic acid, the other from a liver monophosphoinositide) showed growth promoting activity. The phytic acid was only 2 to 4 per cent as effective as the equivalent amount of free inositol; the two monophosphates were about 15 and 70 per cent as effective, respectively. Also, hexachlorocyclohexane was tested as an antagonist of myo-inositol. It was without effect in concentrations many-fold that of inositol. It inhibited growth only when provided at levels between 100 and 1000 times that of inositol, an inhibition that was independent of the concentration of inositol.

When inositol was provided at a level of 10^{-6} M. most of the cell lines tested grew optimally, 5×10^{-7} M. being required for half-maximal growth. These levels are higher than those required for most of the

other members of the vitamin B complex except choline, and they suggest, as the authors point out, that inositol and choline probably serve as metabolites in the synthesis of inositol phosphatides rather than as precursors of coenzymes like the other B-vitamins.

The observation that inositol is an essential growth factor for eight lines of normal human cells is difficult to reconcile, from a general nutritional viewpoint, with the observation that the young rat can synthesize inositol (*Nutrition Reviews* **14,** *219 (1956)*). The authors, in discussing this point, mention several possible explanations: (1) man and the rat may differ in their ability to synthesize inositol; (2) inositol may be synthesized only in certain tissues other than those studied; (3) cells in tissue culture may lose certain synthetic capacities; (4) the rapid growth rate of cells in tissue culture may increase the requirement for inositol well beyond that of cells in the animal body. It is worthy of note that in animal experiments deficiency symptoms responding to inositol have usually been seen in young rapidly growing animals and that spontaneous cures have been reported in longer experiments (*Nutrition Reviews* **2,** *163 (1944)*). There may be a hint here that the young animal cannot synthesize myo-inositol in amounts adequate to satisfy the tissue requirements during the period of rapid growth.

It is to be hoped that these observations on the essentiality of inositol for a variety of cell lines grown in tissue culture will stimulate interest in a reappraisal of the role of inositol in animal nutrition. Diets that are more adequate and more highly purified than those used in the earlier studies on the nutritional significance of inositol are now available. However, techniques which will make possible an estimate of the relative importance of the tissues and the intestinal flora in the synthesis of inositol remain to be developed and exploited. Experiments with germ-free rats have provided some evidence suggesting a major role for the tissues (Luckey *et al.*, *J. Nutrition* **57,** *169 (1955)*) but much remains to be done.

VITAMIN-SPARING ACTION OF SORBITOL

The almost exclusive concern of thiamine for carbohydrate metabolism has been demonstrated by the ability of experimental animals to subsist on diets almost devoid of this vitamin provided carbohydrate is also low or absent (G. G. Banerji, *Biochem. J.* **34,** *1329 (1940)*); **35,** *1354 (1941)*). Even in the absence of typical thiamine deficiency symptoms, however, such animals have impaired ability to oxidize pyruvate, and in this state, metabolizable carbohydrate and pyruvate are quite toxic (Banerji and J. Yudkin, *Biochem. J.* **36,** *530 (1942)*; *Nutrition Reviews* **9,** *357 (1951)*).

Recently, T. B. Morgan and Yudkin (*Nature* **180,** *543 (1957)*) attempted to use this knowledge to clarify the role of sorbitol (a sugar alcohol related to glucose and fructose) as a metabolizable carbohydrate (*Nutrition Reviews* **12,** *178 (1954)*; **14,** *236 (1956)*). Rats were fed a carbohydrate-free, thiamine-deficient diet containing 20 per cent sorbitol. They not only survived (as did those receiving no sorbitol) but they gained weight normally and appeared quite healthy. If the sorbitol were replaced by glucose, the rats developed deficiency symptoms and died in five to ten weeks.

The authors consider that the thiamine-sparing effect of sorbitol is due to its promotion of intestinal synthesis of thiamine by intestinal microflora. In support of this idea the following evidence is cited: a sudden addition of sorbitol to the diet produces severe diarrhea; rats on diets containing

sorbitol had enlarged caeca and increased caecal contents; even on the thiamine-free diet, the tissues of rats on the sorbitol-containing diets contained more thiamine than did those on the glucose-containing diets. If this is the correct explanation, then increased synthesis of other B vitamins might also be promoted by the same means. That this is the case was shown by experiments in which all the B vitamins were excluded from the diet. When the diet contained 20 per cent sorbitol, the rats grew normally in experiments which had continued through three months when reported. Moreover, addition of sorbitol to the vitamin-free diet after cessation of growth brought about renewed growth to normal levels.

Further reports on this subject can be expected to include an explanation of the effect of sorbitol on the microflora of the rat caecum and intestines and the results of similar experiments on human subjects. Considerably more study will have to be devoted to the metabolism of sorbitol and similar substances, however, before many conclusions can be reached regarding the practicability of this finding.

NOTES

Carotene, 20,000 Years Old

One of the problems of the manufacturer of mixed rations for farm animals is that the β-carotene and other valuable carotenoids present at the time of mixing the ingredients may be reduced to a very significant extent during the month or so of storage before use. Even in a single feed ingredient, such as dehydrated lucerne, there may be a virtual disappearance of carotene within a year if storage conditions are not all that they should be.

In contrast to this is the recent finding of J. R. Vallentyne (*Arch. Biochem. Biophys.* **70,** *29* (*1957*)), who has demonstrated the existence of β-carotene molecules that must be presumed to have survived for 20,000 years. This finding was made during the systematic investigation of the organic compounds in the fossil sediment layers from Searles Lake, California. The sample examined was orange-green in color and had a sweet smell. Analysis showed about 30 per cent dry matter, of which one quarter was organic. Extraction with successive solvents yielded 28 mg. of carotenes and 23 mg. of xanthophylls per kilogram of the original dry matter. Using paper chromatography to investigate the carotene fraction, both α- and β-carotene were identified. Another component was probably echinenone (myxoxanthin).

Further extraction of the sediment showed the presence of green pigments, and the spectrum of a methanol extract suggested the presence of chlorophylls. However, the absence of absorption in the region from 700 to 800 mμ indicated the absence of bacteriochlorophyll-like pigments.

The compounds found are of course associated with the type of plants, algae, and molluscs that might have been expected to flourish in the lake. Their survival over such a long period must be attributed to the reducing nature of their environment in the sediment.

Folacin and Histidine Biosynthesis

It has been shown that the number 2 carbon of the imidazole ring of histidine can serve as a formate precursor, and that folacin derivatives are involved in this reaction (*Nutrition Reviews* **15,** *159* (*1957*)). Other work has indicated that in histidine biosynthesis this carbon may be derived from formate (L. Levy and M. J. Coon, *J. Biol. Chem.* **192,** *807* (*1951*)). It then became of interest to determine if the latter reaction requires folacin coenzymes.

H. P. Broquist (*Arch. Biochem. Biophys.* **70,** *210 (1957)*) approached this problem utilizing microorganisms. The addition of small quantities of the folacin antagonist aminopterin to the growth medium of *Torula cremoris* inhibited growth and this inhibition was relieved by methionine. In the presence of methionine larger quantities of aminopterin inhibited growth of the microorganism and the inhibition was relieved by adenine. When the growth medium contained both methionine and adenine, still larger quantities of aminopterin inhibited growth and histidine relieved this growth inhibition. It would then appear that in this microorganism derivatives of folacin are concerned in the synthesis of methionine, purines and histidine.

In experiments with *Lactobacillus arabinosus* aminopterin was found to be less inhibitory in the presence of histidine than in its absence.

These experimental results implicate folacin in histidine biosynthesis. The most plausible explanation is that coenzymes derived from the vitamin are necessary for the transfer of the one-carbon fragment into the number 2 carbon of the imidazole ring of histidine.

Gordon Research Conferences

The Gordon Research Conferences for 1958 will be held from June 9 to August 29 at Colby Junior College (New London, New Hampshire), New Hampshire School (New Hampton, New Hampshire), and Kimball Union Academy (Meridan, New Hampshire).

Topics scheduled for discussion at the Food and Nutrition Conferences to be held at Colby Junior College include the following:

August 11—Amino acid balance; vegetable proteins in human nutrition; physiological aspects of hunger.

August 12—Fatty acid nutrition; body composition changes under caloric restriction; hormonal relationships to nutrition.

August 13—Food supplementation; the food protection program; nutritional factors in congenital malformations; the relationship between nutrition and behavior.

August 14—The establishment of nutritional requirements; nutritional surveys in the Near and Far East; the WHO nutrition program.

August 15—Effect of gibberellin on plant growth and composition; effects of fertilizers on plant nutritional value.

Requests for attendance at the Conferences or for any additional information should be addressed to W. George Parks, Director, Department of Chemistry, University of Rhode Island, Kingston, Rhode Island.

Recent Nutrition Books

Prevention of Chronic Illness. Vol. I. Commission on Chronic Illness. Published for The Commonwealth Fund. Harvard Univ. Press, Cambridge, Mass. Pp. 323. Price $6.00.

Chemical Composition of South Pacific Foods. An annotated Bibliography (Technical Paper No. 100). F. E. Peters. South Pacific Commission, New Caledonia. Pp. 104.

Water Fluoridation: Facts, Not Myths. Louis I. Dublin. Public Affairs Pamphlet No. 251. Public Affairs Pamphlets, 22 East 38th Street, New York 16, N. Y. Price $ .25.

Biochemical Preparations. Vol. V. David Shemin, Editor-in-Chief. John Wiley & Sons, Inc. 1957. Pp. 115.

Chemistry of Natural Food Flavors: A Symposium. Advisory Board on Quartermaster Research and Development Committee on Foods, May, 1957. Pp. 200. Available from the Quartermaster Food and Container Institute for the Armed Forces 1819 W. Pershing Road, Chicago 9, Illinois.

Calorie Requirements. Report of the Second Committee on Calorie Requirements. FAO Nutritional Studies, No. 15. Food & Agriculture Organization of the United Nations. Rome 1957. Pp. 68.

Chemical Anthropology. Icie G. Macy & Harriet J. Kelly. University of Chicago Press. Pp. 143. Price $3.75.

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